CompCytogen 13(2): 105–120 (2019)
doi: 10.3897/CompCytogen.v13i2.33764
http://compcytogen.pensoft.net

RESEARCH ARTICLE

COMPARATIVE Cytogenetics
A peer-reviewed open-access journal
International Journal of Plant & Animal Cytogenetics, Karyosystematics, and Molecular Systematics

Comparative cytogenetics among *Leporinus friderici* and *Leporellus vittatus* populations (Characiformes, Anostomidae): focus on repetitive DNA elements

Thais Aparecida Dulz[1,2], Carla Andrea Lorscheider[2], Viviane Demetrio Nascimento[1], Rafael Bueno Noleto[2], Orlando Moreira-Filho[3], Viviane Nogaroto[4], Marcelo Ricardo Vicari[1,4]

1 *Programa de Pós-Graduação em Genética, Universidade Federal do Paraná, Centro Politécnico, Jardim das Américas, 81531-990, Curitiba, Paraná State, Brazil* **2** *Departamento de Ciências Biológicas, Universidade Estadual do Paraná, Praça Coronel Amazonas, s/n°, 84600-185, União da Vitória, Paraná State, Brazil* **3** *Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, Km 235, 13565-905, São Carlos, São Paulo State, Brazil* **4** *Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Av. Carlos Cavalcanti, 4748, 84030-900, Ponta Grossa, Paraná State, Brazil*

Corresponding author: *Marcelo Ricardo Vicari* (vicarimr@uepg.br)

Academic editor: *I. Bakloushinskaya* | Received 8 February 2019 | Accepted 25 March 2019 | Published 5 April 2019

http://zoobank.org/4B5C985A-3910-445A-8D06-3B82965E6080

Citation: Dulz TA, Lorscheider CA, Nascimento VD, Noleto RB, Moreira-Filho O, Nogaroto V, Vicari MR (2019) Comparative cytogenetics among *Leporinus friderici* and *Leporellus vittatus* populations (Characiformes, Anostomidae): focus on repetitive DNA elements. Comparative Cytogenetics 13(2): 105–120. https://doi.org/10.3897/CompCytogen.v13i2.33764

Abstract

Anostomidae are a neotropical fish family rich in number of species. Cytogenetically, they show a conserved karyotype with 2n = 54 chromosomes, although they present intraspecific/interspecific variations in the number and chromosomal location of repetitive DNA sequences. The aim of the present study was to perform a comparative description of the karyotypes of two populations of *Leporinus friderici* Bloch, 1794 and three populations of *Leporellus vittatus* Valenciennes, 1850. We used conventional cytogenetic techniques allied to fluorescence *in situ* hybridization, using 18S ribosomal DNA (rDNA) and 5S rDNA, a general telomere sequence for vertebrates (TTAGGG)n and retrotransposon (RTE) *Rex1* probes. The anostomids in all studied populations presented 2n = 54 chromosomes, with a chromosome formula of 32m + 22sm for *L. friderici* and 28m + 26sm for *L. vittatus*. Variations in the number and location of the 5S and 18S rDNA chromosomal sites were observed between *L. friderici* and *L. vittatus* populations and

Copyright *Thais Aparecida Dulz et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

species. Accumulation of *Rex1* was observed in the terminal region of most chromosomes in all populations, and telomere sequences were located just on all ends of the 54 chromosomes in all populations. The intraspecific and intergeneric chromosomal changes occurred in karyotype differentiation, indicating that minor chromosomal rearrangements had present in anostomid species diversification.

Keywords
Chromosomal differentiation, karyotype evolution, ribosomal DNA, retrotransposon

Introduction

Eukaryotic chromosomes can be classified into different DNA classes: single copy DNA, which are sequences found only once in a genome; and repetitive DNA, which are sequences repeated from a few tens to millions of times (Sumner 2003). Repetitive DNA can be classified into tandem repeats (multigene families and satellite, minisatellite, and microsatellite DNA) and transposable elements (TEs): transposons and retrotransposons with dispersed distribution in genomes (Sumner 2003).

Satellite DNA and TEs are responsible for a large part of the structural and functional organization of genomes (Sumner 2003, Feschotte 2008), and carry sequences containing DNA double-strand break hotspots, resulting in chromosome/genome reshuffle (Eichler and Sankoff 2003, Longo et al. 2009, Farré et al. 2011, Barros et al. 2017a, Glugoski et al. 2018). The movement of repetitive sequences within the genome promotes chromosomal differentiation, which has an important role on karyotype evolution (Wichman et al. 1991, Pucci et al. 2016, 2018a, 2018b, Lorscheider et al. 2018, do Nascimento et al. 2018).

Anostomids are neotropical fishes with a high number of species and diverse morphology (Garavello and Britski 2003, Graça and Pavanelli 2007, Britski et al. 2012, Ramirez et al. 2017a). Cytogenetically they present a conserved diploid number (2n) of 54 chromosomes, with mostly metacentric (m) and submetacentric (sm) chromosomes (Galetti Jr and Foresti 1986, Galetti Jr et al. 1991, 1995, Venere et al. 2004). Anostomidae species present differentiated karyotypes regarding the distribution of heterochromatin and repetitive sequences, presenting different localizations of heterochromatic bands and repetitive DNA sites (Martins and Galetti Jr 1999, Parise-Maltempi et al. 2007, Porto-Foresti et al. 2008, Hashimoto et al. 2009, Marreta et al. 2012, Borba et al. 2013).

Therefore, although they retain 2n = 54 chromosomes, anostomids present very high intra- and interspecific chromosomal/genetic variability, which is highly compatible with restricted gene flow (Parise-Maltempi et al. 2007, 2013, Ramirez et al. 2017a, 2017b, Silva-Santos et al. 2018). With the aim of better understanding the intra- and interspecific chromosomal differentiation due to accumulation of repetitive sequences, in the present study we performed a comparative evaluation of the karyotypes of two populations of *Leporinus friderici* (Bloch, 1794) and three populations of *Leporellus vittatus* (Valenciennes, 1850). Cytogenetic analysis was performed using Giemsa staining and C-banding, and chromosome mapping of repetitive DNAs using the ribosomal DNA (rDNA) 18S and 5S rDNA, the (TTAGGG)n sequence and the retrotransposon (RTE) *Rex1*.

Material and methods

Specimens of *Leporinus friderici* and *Leporellus vittatus* were collected from rivers belonging to different Brazilian hydrographic basins (Table 1). Fish capture was authorized by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio – license numbers 10538-1 and 15117-1) and the processing was performed in accordance with the Ethical Committee on Animal Use (CEUA 29/2016) of the Universidade Estadual de Ponta Grossa and current Brazilian legislation. The analyzed specimens were identified by taxonomists experts in the Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (Nupelia) museum, Universidade Estadual de Maringá (UEM).

Genomic DNA was extracted from the liver tissue, using the protocol of Doyle and Doyle (1990), from the *Megaleporinus obtusidens* (Ramirez et al. 2017a), described first time in the literature as *Leporinus obtusidens* (Valenciennes, 1837). The 18S rDNA amplification was performed using primers 18S Fw (5'-ccgctttggtgactcttgat-3') and 18S Rv (5'-ccgaggacctcactaaacca-3'), according to Gross et al. (2010). The 5S rDNA sequence was amplified using primers 5SA (5'-tcaaccaaccacaaagacattggcac-3') and 5S (5'-tagacttctgggtggccaaaggaatca-3'), according to Martins and Galetti (1999). The vertebrate telomere sequence (TTAGGG)n was obtained according to Ijdo et al. (1991). The non-long terminal repeats retrotransposon (non-LTR RTE) *Rex1* sequence was obtained by PCR using primers RTX1-F1 Fw (5'-ttctccagtgccttcaacacc-3') and RTX1-R1 Rv (5'-tccctcagcagaaagagtctgctc-3'), according to Volff et al. (1999, 2000). The sequences of the 5S rDNA, 18S rDNA and *Rex-1* were analyzed and their nucleotide identities were confirmed using BLASTn (National Center for Biotechnology Information) and the CENSOR tool for repeated sequences (Kohany et al. 2006). Finally, the sequences were deposited in GenBank (Sequences ID: MH697559, MH701851, MH684488, respectively).

Mitotic chromosomes were obtained according to Blanco et al. (2012) and stained with 5% Giemsa in phosphate buffer, pH 6.8. Heterochromatin detection was performed according to Sumner (1972), with modifications (Lui et al. 2009).

Table 1. Cytogenetic data of *Leporinus friderici* and *Leporellus vittatus* analyzed in the present study. SP = São Paulo State, PR = Paraná State, MG = Minas Gerais State, MT = Mato Grosso State, 2n = diploid number, FN = fundamental number, KF = karyotype formula, term = terminal sites.

Species	River/Basin/State/GPS	2n	FN	KF	5S sites	18S sites	*Rex1*
Leporinus friderici	Mogi-Guaçu River, Upper Paraná Basin – SP (21°58'52"S, 47°17'36"W)	54	108	32m+22sm	pairs 10 and 11	pair 1	term
	Jangada River, Iguaçu River Basin – PR (26°13'5.22"S, 51°16'17.40"W)	54	108	32m+22sm	pairs 3 and 11	pair 1	term
Leporellus vittatus	Mogi-Guaçu River, Upper Paraná Basin – SP (21°58'52"S, 47°17'36"W)	54	108	28m+26sm	pair 3	pair 5	term
	Aripuanã River, Aripuanã River Basin – MT (10°09'57.8"S, 59°26'54.9"W)	54	108	28m+26sm	pairs 6 and 8	pair 6	term
	São Francisco River, São Francisco Basin – MG (20°16'15"S, 45°55'39"W)	54	108	28m+26sm	pair 3	pair 6	term

The 18S rDNA was labeled with digoxigenin-11-dUTP, using the DIG-Nick Translation Mix (Roche Applied Science), according to the manufacturer's recommendations. The 5S rDNA sequence was labeled with biotin 16-dUTP by PCR, and *Rex1* and (TTAGGG)n sequences with digoxigenin-11-dUTP by PCR. PCR reactions were performed with 20 ng DNA template, 1× polymerase reaction buffer, 1.5 mM MgCl2, 40 μM dATP, dGTP and dCTP, 28 μM dTTP, 12 μM digoxigenin-11-dUTP or biotin 16 dUTP, 1 μM of each primer and 1 U of DNA Taq polymerase. The PCR program consisted of an initial step of denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 56 °C for 45 s, 72 °C for 2 min, and a final extension at 72 °C for 7 min.

The general protocol for FISH (Pinkel et al. 1986) followed under hybridization mixture (2.5 ng/μl probe, 50% formamide, 2×SSC, 10% dextran sulfate, at 37 °C for 16 h). Post-hybridization washes were performed in high stringency [50% formamide at 42 °C for 10 min (twice times), 0.1×SSC at 60 °C for 5 min (three times), and 4×SSC 0.05% Tween at room temperature for 5 min (two baths)]. Streptavidin Alexa Fluor 488 (Molecular Probes) and Anti-digoxigenin rhodamine fab fragments (Roche Applied Science) antibodies were used for probes detection. The chromosomes were stained with DAPI (0.2 μg/ml) in Vectashield mounting medium (Vector) and analyzed under epifluorescence microscopy.

Chromosome preparations were analyzed using the brightfield and epifluorescence microscope Zeiss Axio Lab 1, coupled to the Zeiss AxioCam ICM1 camera with the Zen Lite software and a resolution of 1.4 megapixels (Carl Zeiss). The karyotypes were organized and classified as metacentric (m) or submetacentric (sm) according to Levan et al. (1964).

Results

All anostomids evaluated in the present study presented 2n = 54 chromosomes and a fundamental number (FN) of 108 (Table 1). The two populations of *L. friderici* (Mogi–Guaçu and Jangada rivers) presented a karyotype formula (KF) of 32m + 22sm (Fig. 1a, b), and the three populations of *L. vittatus* (Mogi–Guaçu, Aripuanã and São Francisco rivers) a karyotype formula of 28m + 26sm (Fig. 1c, d, e). Sex chromosome heteromorphism was not detected in the populations/species analyzed.

C-banding showed discrete blocks of centromeric heterochromatin for *L. friderici*, with very evident blocks in the terminal regions of the long arms of just one homologue of chromosomes 1 and 5 for the population of the Mogi–Guaçu river (Fig. 2a); and, in the subterminal regions of pairs 1 and 17 for the population of the Jangada river (Fig. 2b). *Leporellus vittatus* showed blocks of heterochromatin in the pericentromeric or proximal regions of most chromosomes (Fig. 2c, d), which was very evident for the populations from the Mogi–Guaçu and Aripuanã rivers and less evident for the populations from the São Francisco river (Fig. 2e).

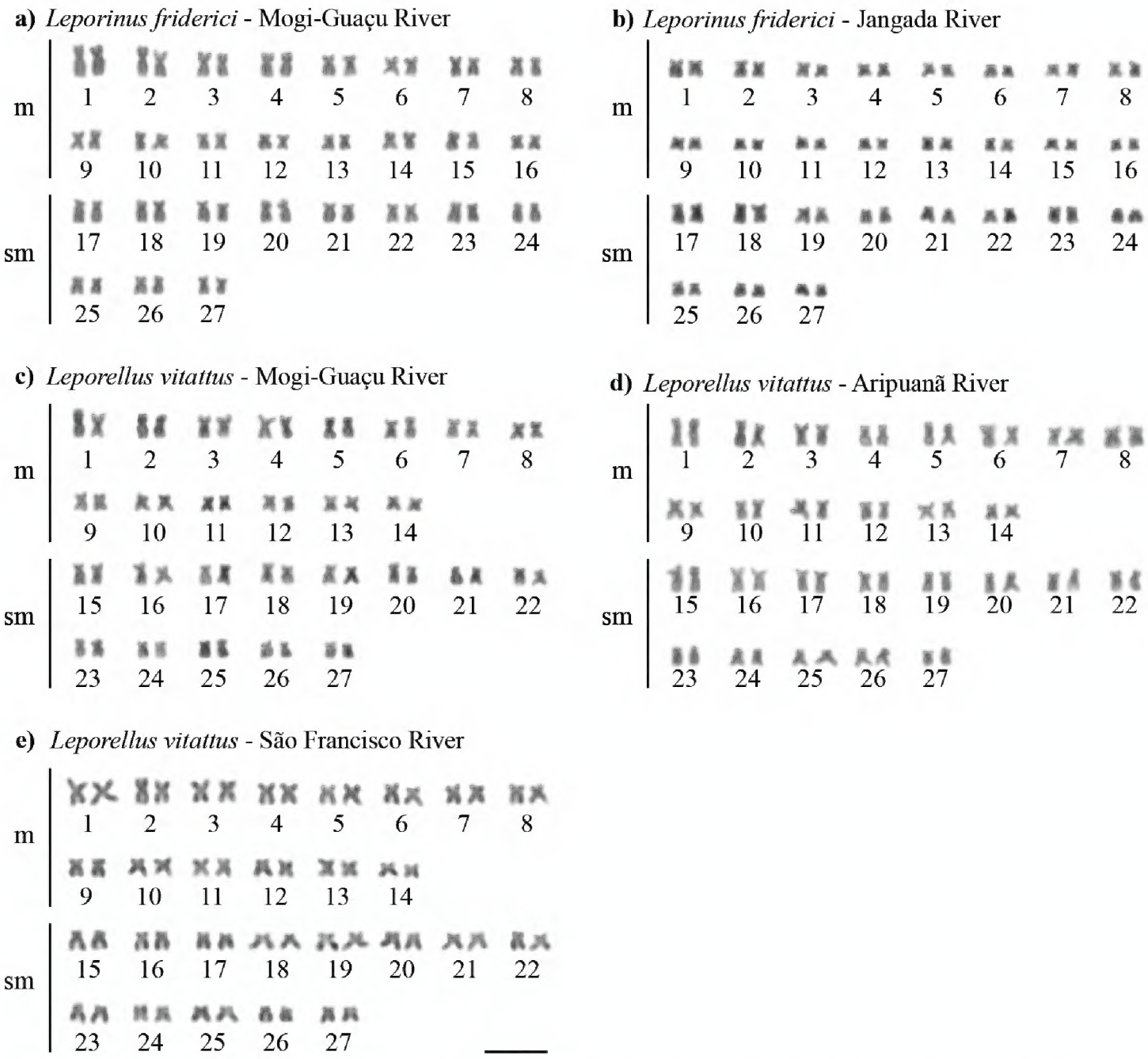

Figure 1. Karyotypes of *Leporinus friderici* (**a, b**) and *Leporellus vittatus* (**c, d, e**) after conventional Giemsa staining. Scale bar: 10 μm.

Double-FISH using 5S and 18S rDNA probes detected one 45S rDNA site in the short arm (p) of chromosome pair 1 for both populations of *L. friderici* (Fig. 3a, b). The 5S rDNA was located in the pericentromeric region of chromosome pair 10 and in the short arm (p) of pair 11 for *L. friderici* from the Mogi–Guaçu river (Fig. 3a), whereas it was located in the p arm of chromosome pairs 3 and 11 for *L. friderici* from the Jangada river (Fig. 3b). *Leporellus vittatus* from the Mogi–Guaçu river presented 45S rDNA in the terminal region of the long arm (q) of pair 5, and 5S rDNA was located in the proximal region of 3p pair (Fig. 3c). In *L. vittatus* from the Aripuanã river, the 45S rDNA was located in synteny with 5S rDNA in the chromosome pair 6, with terminal location 6q for 45S rDNA and proximal q arm site for 5S rDNA, and an additional 5S rDNA site in the proximal q arm of pair 8 (Fig. 3d). *Leporellus vittatus* from the São Francisco river presented the 45S rDNA in the terminal region of 6q, and the 5S

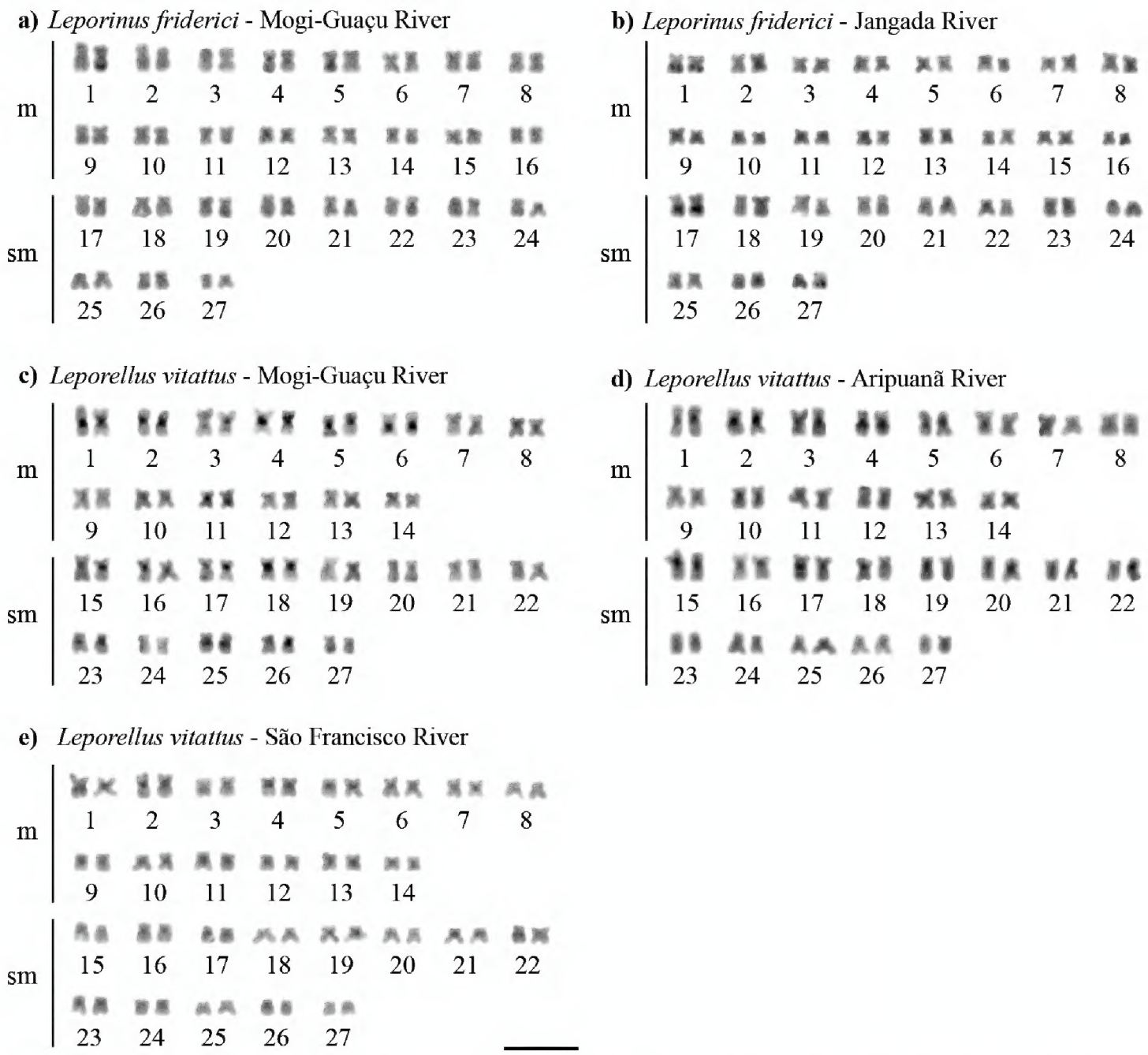

Figure 2. Karyotypes of *Leporinus friderici* (**a, b**) and *Leporellus vittatus* (**c, d, e**) after C-banding. Scale bar: 10 μm.

rDNA in the proximal region of 3p (Fig. 3e). *In situ* mapping of RTE *Rex1* (Fig. 4a–e) and (TTAGGG)n (Fig. 5a–e) showed signals in the terminal regions of all chromosomes for all populations of both *L. friderici* and *L. vittatus*. In *L. vittatus* from the Mogi-Guaçu river, the telomeres signals were tiny in all metaphases analyzed (Fig. 5c).

Discussion

The present cytogenetic analysis confirmed the conservation of the karyotype macrostructure of 2n = 54 chromosomes in *Leporinus friderici* and *Leporellus vittatus*, with metacentric and submetacentric chromosomes (FN=108). This karyotype structure is shared by most species belonging to Anostomidae (Galetti Jr et al. 1995, Venere et al. 2004). In addition, *L. friderici* and *L. vittatus* presented small differences in their karyo-

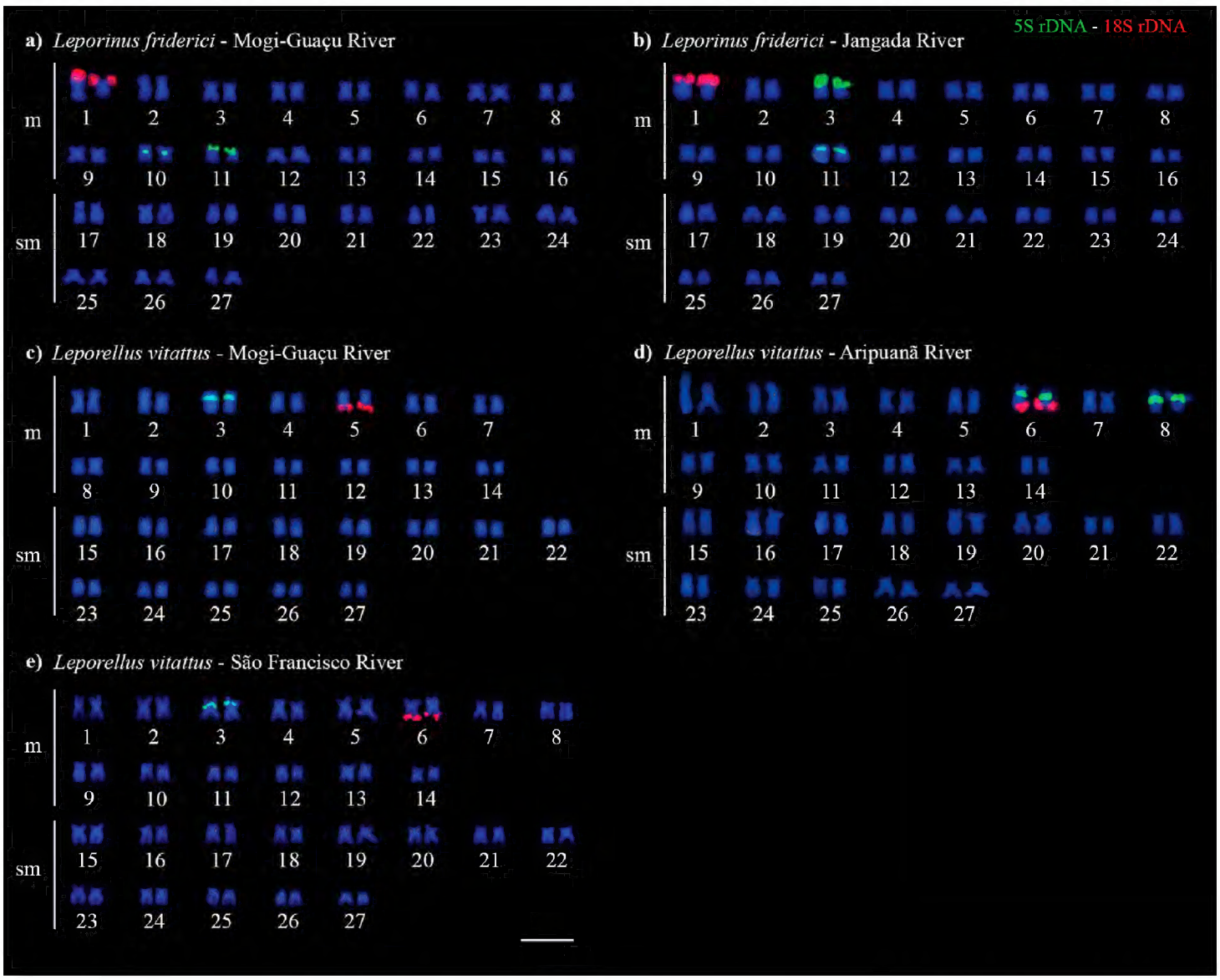
5S rDNA - 18S rDNA
a) Leporinus friderici - Mogi-Guaçu River
b) Leporinus friderici - Jangada River
c) Leporellus vittatus - Mogi-Guaçu River
d) Leporellus vittatus - Aripuanã River
e) Leporellus vittatus - São Francisco River
m
sm

Figure 3. Karyotypes of *Leporinus friderici* (**a, b**) and *Leporellus vittatus* (**c, d, e**) submitted to fluorescence *in situ* hybridization with 18S rDNA and 5S rDNA probes. Scale bar: 10 μm.

type formulas resulted of the chromosome rearrangements such as pericentric inversions, translocations or centromere repositioning, which alters the chromosome morphology without any accompanying chromosomal rearrangements (Rocchi et al. 2012).

Some chromosomal markers presented some differentiation within and between species of anostomids. Intraspecific variations were observed in the chromosomal location and quantity of heterochromatin blocks, which were mainly located in pericentromeric regions in *L. vittatus* and terminal positions of chromosomes in *L. friderici*. These heterochromatin distribution in the chromosomes have also been observed for other anostomids (Pereira et al. 2002, Aguilar and Galetti Jr 2008, Barros et al. 2017b). Satellite DNA is one of the components of heterochromatin, which is also enriched in other dispersed repeated elements, including transposons (Mazzuchelli and Martins 2009, Vicari et al. 2010). It is usually accepted that the number of repetitive copies of a heterochromatin block may increase through mechanisms of homologous recombination, TEs invasion, or replication slippage for microsatellite expansion inside heterochromatin (Gray 2000, Kantek et al. 2009, Kelkar et al. 2011, Glugoski et al. 2018). These mechanisms may play a role in the microstructural differentiation

a) Leporinus friderici - Mogi-Guaçu River
b) Leporinus friderici - Jangada River
Rex1
c) Leporellus vitattus - Mogi-Guaçu River
d) Leporellus vitattus - Aripuanã River
e) Leporellus vitattus - São Francisco River
m
sm

Figure 4. Karyotypes of *Leporinus friderici* (**a, b**) and *Leporellus vittatus* (**c, d, e**) submitted to fluorescence *in situ* hybridization with *Rex1* probe. Scale bar: 10 μm.

of heterochromatin chromosome blocks once no evident large heterochromatic blocks were observed in anostomids species analyzed.

In situ location of ribosomal genes showed that these sites were also involved in the chromosomal changes, especially in the studied *L. vittatus* populations. The location of rDNA in different positions and number of chromosomal sites also supports the hypothesis of population differentiation. On the other hand, the location of rDNA sites was observed to be highly conserved in the karyotypes of some anostomids (Martins and Galetti Jr 1999, 2000, 2001). In the present study, consistent differences in the location of rDNA sites were observed between the *L. vittatus* populations evaluated. These differences are exclusive conditions due to population isolation and contribute to genomic diversification in this fish group.

Anostomids usually present only one pair of 45S rDNA (Martins and Galetti Jr 1999), being a common characteristic of this group. Previous studies observed polymorphisms in the number of 45S rDNA sites in *Leporinus taeniatus* Lütken, 1875, *Leporinus trifasciatus* Steindachner, 1876, *Rhytiodus microlepis* Kner, 1858 and *Schizodon fasciatus* Spix & Agassiz, 1829 (Barros et al. 2017b). In the present study, although

(TTAGGG)n
a) Leporinus friderici - Mogi-Guaçu River
b) Leporinus friderici - Jangada River
c) Leporellus vittatus - Mogi-Guaçu River
d) Leporellus vittatus - Aripuanã River
e) Leporellus vittatus - São Francisco River
m
sm

Figure 5. Karyotypes of *Leporinus friderici* (**a, b**) and *Leporellus vittatus* (**c, d, e**) submitted to fluorescence *in situ* hybridization with (TTAGGG)n probe. Scale bar: 10 μm.

this was also observed, differences in the chromosomal position of 45S rDNA were additionally observed between species, with signals in the terminal region of the p arm for *L. friderici* and in the q arm for *L. vittatus*. The rDNAs usually present high rates of karyotype rearrangements in evolutionary lineages (Symonová et al. 2013). These sequence movements within karyotypes have been proposed to occur by transposition and/or by transposon-mediated by TEs in a non-homologous recombination mechanism (Symonová et al. 2013, Barros et al. 2017a, Glugoski et al. 2018). The *L. vittatus* specimens from the Aripuanã river presented synteny of 45S rDNA and 5S rDNA, in contrast with the specimens from the Mogi–Guaçu and São Francisco rivers and the *L. friderici* populations corroborating to high evolutionary chromosomal change level to rDNA sites. The rDNA synteny was also observed in other anostomids, such as *L. trifasciatus*, *S. fasciatus* and *Laemolyta taeniata* (Kner, 1858), showing that it is a recurrent chromosomal characteristic of this group (Barros et al. 2017b).

Recently, some studies have proposed that the dispersal of ribosomal sites and changes in their chromosomal location may affect recombination rates in these specific sites, and that these changes can lead to rapid genome divergence (Symonová

et al. 2013). Therefore, these populational chromosome rearrangements due to rDNA transposition could promote differentiation (Symonová et al. 2013, Pucci et al. 2014, Barbosa et al. 2017), which may lead to speciation, as observed in the present study for Anostomidae.

The chromosomal mapping of the non-LTR retrotransposon family *Rex* (*Rex1*, *Rex3* and *Rex6*) has been conducted in the genomes of different teleost species (Volff et al. 1999, 2000, Cioffi et al. 2010, Valente et al. 2011, Borba et al. 2013, Sczepanski et al. 2013, among others). Although they may have a dispersed distribution (Ozouf-Costaz et al. 2004), in most cases, they show strong association with heterochromatic regions (Cioffi et al. 2010, Valente et al. 2011). Overall, the accumulation of RTE sequences in the terminal region of chromosomes has been well documented in *Drosophila melanogaster* (Meigen, 1830) and in *Sorubim lima* (Bloch & Schneider, 1801), a Neotropical catfish (Eickbush and Furano 2002, Sczepanski et al. 2013). The distribution of *Rex1* sequences in terminal regions of chromosomes in some species of Anostomidae was also detected by *in situ* mapping (Borba et al. 2013). Transpositions and DNA repair by non-homologous recombination involving repetitive sequences in the terminal regions of chromosomes are common during the Rabl configuration of cell division (Schweizer and Loid 1987, Sumner 2003). Furthermore, an efficient strategy to limit the damage caused by retrotransposition in the host genome is to direct the insertion in fairly safe regions, poor in genes, for example in heterochromatin or at telomeres (Okazaki et al. 1995, Zou et al. 1996, Takahashi et al. 1997).

Telomere shortening is usually prevented by telomerase, a reverse transcriptase which adds telomeric repeats to the chromosome ends, thus elongating telomeres (Makarov et al. 1997). The phylogeny involving telomerases and retrotransposons was confirmed after the discovery of a group of retrotransposons, called elements like Penelope, which encodes reverse transcriptase (RT) directly related to an enzyme telomerase (Arkhipova et al. 2003). In *Drosophila*, retrotransposons protect the ends of chromosomes, due to the absence of telomerase, which was possibly lost during evolution (Biessmann et al. 1990). TEs can play a role in the reorganization of the genome being co-opted or exapted to form new genomic functions (Feschotte 2008). This observation suggests the versatility of RT activity in counteracting the chromosome shortening associated with genome replication and that retrotransposons can provide this activity in case of a dysfunctional telomerase. In anostomids analyzed, the (TTAGGG) n sequence was detected in the chromosomal ends, indicating telomerase activity. The short telomere signals detected in *L. vittatus* from the Mogi-Guaçu population can be resulted of the somatic cells telomere shortens with each cell division or, due to inconsistent FISH detection in short telomere sequences. Finally, we observed absence of an interstitial telomeric sequence (ITS), together with the conserved karyotype of 2n = 54 chromosomes, indicating that just non-Robertsonian events may play a role in karyotype diversification in the studied species.

The present study showed intraspecific karyotype variation in populations with isolation of gene flow, and interspecific variation between populations of *L. friderici* and *L. vittatus*. This can be partly explained by genome reorganization due to move-

ment of heterochromatin blocks, ribosomal sites, satellite repetitive sequences, and transposable elements. Our results therefore confirm the conservation of the chromosome macrostructure and indicate karyotypic differentiation at the microstructural level during evolution in Anostomidae.

Acknowledgments

The authors are grateful to the Ministério do Meio Ambiente / Instituto Chico Mendes de Conservação da Biodiversidade (MMA / ICMBio license numbers 10538-1 e 15117) for authorizing the capture of the fish species. The study received funding from the Coordenadoria de Aperfeiçoamento de Ensino Superior (CAPES - Finance Code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Secretaria de Ciência e Tecnologia do Estado do Paraná (SETI) and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Fundação Araucária).

References

Aguilar CT de, Galetti Jr PM (2008) Chromosome mapping of 5S rRNA genes differentiates Brazilian populations of *Leporellus vittatus* (Anostomidae, Characiformes). Genetics and Molecular Biology 31: 188–194. https://doi.org/10.1590/S1415-47572008000200004

Arkhipova IR, Pyatkov KI, Meselson M, Evgen'ev MB (2003) Retroelements containing introns in diverse invertebrate taxa. Nature Genetics 33: 123–124. https://doi.org/10.1038/ng1074

Barbosa P, Pucci MB, Nogaroto V, Almeida MC, Artoni RF, Vicari MR (2017) Karyotype analysis of three species of *Corydoras* (Siluriformes: Callichthyidae) from southern Brazil: rearranged karyotypes and cytotaxonomy. Neotropical Ichthyology 15: e160056. https://doi.org/10.1590/1982-0224-20160056

Barros AV, Wolski MAV, Nogaroto V, Almeida MC, Moreira-Filho O, Vicari MR (2017a) Fragile sites, dysfunctional telomere and chromosome fusions: What is 5S rDNA role? Gene 608: 20–27. https://doi.org/10.1016/j.gene.2017.01.013

Barros LC, Galetti Jr PM, Feldberg E (2017b) Mapping 45S and 5S ribosomal genes in chromosomes of Anostomidae fish species (Ostariophysi, Characiformes) from different Amazonian water types. Hydrobiologia 789: 77–89. https://doi.org/10.1007/s10750-015-2583-8

Biessmann H, Mason JM, Ferry K, d'Hulst M, Valgeirsdottir K, Traverse KL, Pardue ML (1990) Addition of telomere-associated HeT DNA sequences "heals" broken chromosome ends in *Drosophila*. Cell 61: 663–673. https://doi.org/10.1016/0092-8674(90)90478-W

Blanco DR, Bertollo LAC, Lui RL, Vicari MR, Margarido VP, Artoni RF, Moreira-Filho O (2012) A new technique for obtaining mitotic chromosome spreads from fishes in the field. Journal of Fish Biology 81: 351–357. https://doi.org/10.1038/443521a

Borba RS, Lourenço da Silva E, Parise-Maltempi PP (2013) Chromosome mapping of retrotransposable elements Rex1 and Rex3 in *Leporinus* Spix, 1829 species (Characiformes: Anostomidae) and its relationships among heterochromatic segments and W sex chromosome. Mobile Genetic Elements 3: e27460. https://doi.org/10.4161/mge.27460

Britski HA, Birindelli JLO, Garavello JC (2012) A new species of *Leporinus* Agassiz, 1829 from the upper Rio Paraná basin (Characiformes, Anostomidae) with redescription of *L. elongatus* Valenciennes, 1850 and *L. obtusidens* (Valenciennes, 1837). Papéis Avulsos de Zoologia 52: 441–475 https://doi.org/10.1590/S0031-10492012021700001

Cioffi MB, Martins C, Bertollo LAC (2010) Chromosome spreading of associated transposable elements and ribosomal DNA in the fish *Erythrinus erythrinus*. Implications for genome change and karyoevolution in fish. BMC Evolutionary Biology 10: 271. https://doi.org/10.1186/1471-2148-10-271

do Nascimento VD, Coelho KA, Nogaroto V, de Almeida RB, Ziemniczak K, Centofante L, Pavanelli CS, Torres RA, Moreira-Filho O, Vicari MR (2018) Do multiple karyomorphs and population genetics of freshwater darter characines (*Apareiodon affinis*) indicate chromosomal speciation? Zoologischer Anzeiger 272: 93–103. https://doi.org/10.1016/j.jcz.2017.12.006

Doyle JJ, Doyle JL (1990) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Eichler EE, Sankoff D (2003) Structural Dynamics of Eukaryotic Chromosome Evolution. Science 301: 793–797. https://doi.org/10.1126/science.1086132

Eickbush T, Furano AV (2002) Fruit flies and humans respond differently to retrotransposons. Current Opinion in Genetics & Development. 12: 669–674. https://doi.org/10.1016/S0959-437X(02)00359-3

Farré M, Bosch M, López-Giráldez F, Ponsà M, Ruiz-Herrera A (2011) Assessing the role of tandem repeats in shaping the genomic architecture of great apes. PLoS One 6: e27239. https://doi.org/10.1371/journal.pone.0027239

Feschotte C (2008) The contribution of transposable elements to the evolution of regulatory networks. Nature Reviews Genetics 9: 397–405. https://doi.org/10.1038/nrg2337

Galetti Jr PM, Foresti F (1986) Evolution of the ZZ/ZW system in *Leporinus* (Pisces, Anostomidae). Cytogenetic and Genome Research 43: 43–46. https://doi.org/10.1159/000132296

Galetti Jr PM, Cesar ACG, Venere PC (1991) Heterochromatin and NORs variability in *Leporinus* fish (Anostomidae, Characiformes). Caryologia 44: 287–292. https://doi.org/10.1080/00087114.1991.10797193

Galetti Jr PM, Lima NRW, Venere PC (1995) A Monophyletic ZW Sex Chromosome System in *Leporinus* (Anostomidae, Characiformes). Cytologia 60: 375–382. https://doi.org/10.1508/cytologia.60.375

Garavello JC, Britski HA (2003) Family Anostomidae. In: Reis RE, Kullander S, Ferraris Jr CF (Eds) Check list of the freshwater fishes of south and central America. EDIPUCRS, Porto Alegre, 71–84.

Glugoski L, Giuliano-Caetano L, Moreira-Filho O, Vicari MR, Nogaroto V (2018) Co-located hAT transposable element and 5S rDNA in an interstitial telomeric sequence suggest the formation of Robertsonian fusion in armored catfish. Gene 650: 49–54. https://doi.org/10.1016/j.gene.2018.01.099

Graça WJ, Pavanelli CS (2007) Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. Eduem, Maringá, 308 pp.

Gray YHM (2000) It takes two transposons to tango: transposable-element-mediated chromosomal rearrangements. Trends in Genetics 16: 461–68. https://doi.org/10.1016/S0168-9525(00)02104-1

Gross MC, Schneider CH, Valente GT, Martins C, Feldberg E (2010) Variability of 18S rDNA locus among *Symphysodon* fishes: chromosomal rearrangements. Journal of Fish Biology 76: 1117–1127. https://doi.org/10.1111/j.1095-8649.2010.02550.x

Hashimoto DT, Parise-Maltempi PP, Laudicina A, Bortolozzi J, Senhorini JA, Foresti F, Porto-Foresti F (2009) Repetitive DNA probe linked to sex chromosomes in hybrids between Neotropical fish *Leporinus macrocephalus* and *Leporinus elongatus* (Characiformes, Anostomidae). Cytogenetic and Genome Research 124: 151–157. https://doi.org/10.1159/000207523

Ijdo JW, Wells RA, Baldini A, Reeders ST (1991) Improved telomere detection using a telomere repeat probe (TTAGGG) n generated by PCR. Nucleic Acids Research 19: 4780–4780. https://doi.org/10.1093/nar/19.17.4780

Kantek DLZ, Vicari MR, Peres WAM, Cestari MM, Artoni RF, Bertollo LAC, Moreira-Filho O (2009) Chromosomal location and distribution of As51 satellite DNA in five species of the genus *Astyanax* (Teleostei, Characidae, *Incertae sedis*). Journal of Fish Biology 75: 408–421. https://doi.org/10.1111/j.1095-8649.2009.02333.x

Kelkar YD, Eckert KA, Chiaromonte F, Makova KD (2011) A matter of life or death: How microsatellites emerge in and vanish from the human genome. Genome Research 21: 2038–2048. https://doi.org/10.1101/gr.122937.111

Kohany O, Gentles AJ, Hankus L, Jurka J (2006) Annotation, submission and screening of repetitive elements in Repbase: repbase Submitter and Censor. BMC Bioinformatics 7: 474. https://doi.org/10.1186/1471-2105-7-474

Levan A, Fredga K, Sandberg AA (2009) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Longo MS, Carone DM, NISC Comparative Sequencing Program, Green ED, O'Neill MJ, O'Neill RJ (2009) Distinct retroelement classes define evolutionary breakpoints demarcating sites of evolutionary novelty. BMC Genomics 10: 334. https://doi.org/10.1186/1471-2164-10-334

Lorscheider CA, Oliveira JIN, Dulz TA, Nogaroto V, Martins-Santos IC, Vicari MR (2018) Comparative cytogenetics among three sympatric *Hypostomus* species (Siluriformes: Loricariidae): an evolutionary analysis in a high endemic region. Brazilian Archives of Biology and Technology 61: e18180417. https://doi.org/10.1590/1678-4324-2018180417

Lui RL, Blanco DR, Margarido VP, Moreira-Filho O (2009) First description of B chromosomes in the family Auchenipteridae, *Parauchenipterus galeatus* (Siluriformes) of the São Francisco River basin (MG, Brazil). Micron 40: 552–559. https://doi.org/10.1016/j.micron.2009.03.004

Makarov VL, Hirose Y, Langmore JP (1997) Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. Cell 88: 657–666. https://doi.org/10.1016/S0092-8674(00)81908-X

Marreta ME, Faldoni FLC, Parise-Maltempi PP (2012) Cytogenetic mapping of the W chromosome in the genus *Leporinus* (Teleostei, Anostomidae) using a highly repetitive DNA sequence. Journal of Fish Biology 80: 630–637. https://doi.org/10.1111/j.1095-8649.2011.03199.x

Martins C, Galetti Jr PM (1999) Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). Chromosome Research 7: 363–367. https://doi.org/10.1023/A:100921603

Martins C, Galetti Jr PM (2000) Conservative distribution of 5S rDNA loci in *Schizodon* (Pisces, Anostomidae) chromosomes. Chromosome Research 8: 353–355. https://doi.org/10.1023/A:1009243815280

Martins C, Galetti Jr PM (2001) Two 5S rDNA arrays in Neotropical fish species: is it a general rule for fishes? Genetica 111: 439–446. https://doi.org/10.1023/A:1013799516717

Mazzuchelli J, Martins C (2009) Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*. Genetica 136: 461–469 https://doi.org/10.1007/s10709-008-9346-7

Okazaki S, Ishikawa H, Fujiwara H (1995) Structural analysis of TRAS1, a novel family of telomeric repeat-associated retrotransposons in the silkworm, *Bombyx mori*. Molecular and Cellular Biology 15: 4545–4552. https://doi.org/10.1128/MCB.15.8.4545

Ozouf-Costaz C, Brandt J, Koerting C, Pisano E, Bonillo C, Coutanceau JP, Volff JN (2004) Genome dynamics and chromosomal localization of the non-LTR retrotransposons Rex1 and Rex3 in Antarctic fish. Antarctic Science 16: 51–57. https://doi.org/10.1017/S0954102004001816

Parise-Maltempi PP, Martins C, Oliveira C, Foresti F (2007) Identification of a new repetitive element in the sex chromosomes of *Leporinus elongatus* (Teleostei: Characiformes: Anostomidae): new insights into the sex chromosomes of *Leporinus*. Cytogenetic and Genome Research 116: 218–223. https://doi.org/10.1159/000098190

Parise-Maltempi P, da Silva E, Rens W, Dearden F, O'Brien PC, Trifonov V, Ferguson-Smith MA (2013) Comparative analysis of sex chromosomes in *Leporinus* species (Teleostei, Characiformes) using chromosome painting. BMC Genetics 14: 60. https://doi.org/10.1186/1471-2156-14-60

Pereira MA, Oliveira C, Foresti F, Maistro EL (2002) Cytogenetic and Nuclear DNA Content Analysis in Anostomidae Fishes from the Sapucai River, Minas Gerais State, Brazil. Cytologia 67: 289–296. https://doi.org/10.1508/cytologia.67.289

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proceedings of the National Academy of Sciences 83: 2934–2938. https://doi.org/10.1073/pnas.83.9.2934

Porto-Foresti F, Hashimoto DT, Alves AL, Almeida RBC, Senhorini JA, Bortolozzi J, Foresti F (2008) Cytogenetic markers as diagnoses in the identification of the hybrid between Piauçu (*Leporinus macrocephalus*) and Piapara (*Leporinus elongatus*). Genetics and Molecular Biology 31: 195–202. https://doi.org/10.1590/S1415-47572008000200005

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, Foresti F, Moreira-Filho O, Vicari MR (2014) Population differentiation and speciation in the genus *Characidium* (Characiformes: Crenuchidae): effects of reproductive and chromosomal barriers. Biological Journal of the Linnean Society 111: 541–553. https://doi.org/10.1111/bij.12218

Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Scacchetti PC, Pansonato-Alves JC, Foresti F, Moreira-Filho O, Vicari MR (2016) Chromosomal spreading of microsatellites and (TTAGGG)n sequences in the *Characidium zebra* and *C. gomesi* genomes (Characiformes: Crenuchidae). Cytogenetic and Genome Research 149: 182– 190. https://doi.org/10.1159/000447959

Pucci MB, Nogaroto V, Bertollo LAC, Moreira-Filho O, Vicari MR (2018a) The karyotypes and evolution of ZZ/ZW sex chromosomes in the genus *Characidium* (Characiformes, Crenuchidae). Comparative Cytogenetics 12: 421–438. https://doi.org/10.3897/CompCytogen.v12i3.28736

Pucci MB, Nogaroto V, Moreira-Filho O, Vicari MR (2018b) Dispersion of transposable elements and multigene families: Microstructural variation in *Characidium* (Characiformes: Crenuchidae) genomes. Genetics and Molecular Biology 41: 585–592. https://dx.doi.org/10.1590/1678-4685-gmb-2017-0121

Ramirez JL, Birindelli JL, Carvalho DC, Affonso PRAM, Venere PC, Ortega H, Carrillo-Avila M, Rodríguez-Pulido JA, Galetti Jr PM (2017a) Revealing Hidden Diversity of the Underestimated Neotropical Ichthyofauna: DNA Barcoding in the Recently Described Genus *Megaleporinus* (Characiformes: Anostomidae). Frontiers in Genetics 8: 149. https://doi.org/10.3389/fgene.2017.00149

Ramirez JL, Birindelli JLO, Galetti Jr PM (2017b) A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. Molecular Phylogenetics and Evolution 107: 308–323. https://doi.org/10.1016/j.ympev.2016.11.012

Rocchi M, Archidiacono N, Schempp W, Capozzi O, Stanyon R (2012) Centromere repositioning in mammals. Heredity 108: 59–67. https://doi.org/10.1038/hdy.2011.101

Schweizer D, Loid J (1987) A model for heterochromatin dispersion and the evolution of C-band pattern. In: Stahl A, Luciani JM, Vagner-Capodano AM (Eds) Chromosomes Today. Springer, Dordrecht, 61–74. https://doi.org/10.1007/978-94-010-9166-4 7

Sczepanski TS, Vicari MR, de Almeida MC, Nogaroto V, Artoni RF (2013) Chromosomal organization of repetitive DNA in *Sorubim lima* (Teleostei: Pimelodidae). Cytogenetic and Genome Research 141: 309–316. https://doi.org/10.1159/000353845

Silva-Santos R, Ramirez JL, Galetti PM, Freitas PD (2018) Molecular evidences of a hidden complex scenario in *Leporinus* cf. *friderici*. Frontiers in Genetics 9: 47. https://doi.org/10.3389/fgene.2018.00047

Symonová R, Majtánová Z, Sember A, Staaks GB, Bohlen J, Freyhof J, Rábová M, Ráb P (2013) Genome differentiation in a species pair of coregonine fishes: an extremely rapid speciation driven by stress-activated retrotransposons mediating extensive ribosomal DNA multiplications. BMC Evolutionary Biology 13: 42. https://doi.org/10.1186/1471-2148-13-42

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306. https://doi.org/10.1016/0014-4827(72)90558-7

Sumner AT (2003) Chromosomes: organization and function. Blackwell Publishing, Malden, 287 pp.

Takahashi H, Okazaki S, Fujiwara H (1997) A new family of site-specific retrotransposons, SART1, is inserted into telomeric repeats of the silkworm, *Bombyx mori*. Nucleic Acids Research 25: 1578–1584. https://doi.org/10.1093/nar/25.8.1578

Valente GT, Mazzuchelli J, Ferreira IA, Poletto AB, Fantinatti BEA, Martins C (2011) Cytogenetic mapping of the retroelements Rex1, Rex3 and Rex6 among cichlid fish: new insights on the chromosomal distribution of transposable elements. Cytogenetic and Genome Research 133: 34–42. https://doi.org/10.1159/000322888

Venere PC, Alves Ferreira I, Martins C, Galetti Jr PM (2004) A novel ZZ/ZW sex chromosome system for the genus *Leporinus* (Pisces, Anostomidae, Characiformes). Genetica 121: 75–80. https://doi.org/10.1023/B:GENE.0000019936.40970.7a

Vicari MR, Nogaroto V, Noleto RB, Cestari MM, Cioffi MB, Almeida MC, Cioffi MB, Almeida MC, Moreira-Filho O, Bertollo LAC, Artoni RF (2010) Satellite DNA and chromosomes in Neotropical fishes: methods, applications and perspectives. Journal of Fish Biology 76: 1094–1116. https://doi.org/10.1111/j.1095-8649.2010.02564.x

Volff JN, Körting C, Sweeney K, Schartl M (1999) The non-LTR retrotransposon *Rex3* from the fish *Xiphophorus* is widespread among teleosts. Molecular Biology and Evolution 16: 1427–1438. https://doi.org/10.1093/oxfordjournals.molbev.a026055

Volff JN, Körting C, Schartl M (2000) Multiple Lineages of the Non-LTR Retrotransposon Rex1 with Varying Success in Invading Fish Genomes. Molecular Biology and Evolution 17: 1673–1684. https://doi.org/10.1093/oxfordjournals.molbev.a026266

Wichman HA, Payne CT, Ryder OA, Hamilton MJ, Maltbie M, Baker RJ (1991) Genomic distribution of heterochromatic sequences in equids: implications to rapid chromosomal evolution. Journal of Heredity 82: 369–377. https://doi.org/10.1093/oxfordjournals.jhered.a111106

Zou S, Ke N, Kim JM, Voytas DF (1996) The *Saccharomyces* retrotransposon Ty5 integrates preferentially into regions of silent chromatin at the telomeres and mating loci. Genes & Development 10: 634–645. https://doi.org/10.1101/gad.10.5.634